The Journal of Animal & Plant Sciences, 27(6): 2017, Page: 1781-1794
ISSN: 1018-7081

# DIETS CONTAINING SELENIZED YEAST, SELENATE, CARNOSIC ACID AND FISH OIL CHANGE THE CONTENT OF FATTY ACIDS, TOCOPHEROLS AND CHOLESTEROL IN THE SUBCUTANEOUS FAT OF LAMBS

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## ABSTRACT

Male lambs were divided into 5 groups of 6 animals. For 35 days each group was fed one of the following diets: control diet containing 3% rapeseed oil (RO); ROFO diet containing 2% RO and 1% fish oil (FO); CA diet with the addition of 2% RO, 1% FO and 0.1% carnosic acid (CA); CASeY diet with the addition of 0.35 ppm selenium (Se)in the form of selenized-yeast (SeY) to the CA diet; CASeVI diet with the addition of 0.35 ppm Se in the form of selenate (SeVI) to the CA diet. Subcutaneous adipose tissue (SAT) samples were collected from lambs. The experimental diets decreased the C15:0 and C18:0 contents and the sum of conjugated linoleic acid isomers in the SAT when compared to the control diet. The content of long-chain polyunsaturated fatty acids (LPUFA), including n-3LPUFA in the SAT, were most effectively increased by the ROFO diet. The CASeVI diet most effectively increased the content of cholesterol in the SAT. The CASeY and CASeVI diets most effectively increased the content of all tocopherols in the SAT. Oxidative stress in the SAT was most effectively reduced by the CA diet. The ROFO diet increased oxidative stress in the SAT relative to the control diet.

**Key words:** fatty acid; fish oil; carnosic acid; selenium; tocopherols; subcutaneous adipose tissue; lambs.

## INTRODUCTION

Including saturated fatty acids (SFA) of animal origin in human diets may increase the risk of cardiovascular diseases (Joyce *et al.,* 2009). According to Mir *et al.* (2002) oils can be added to the diets of ruminants to change the fatty acid composition of adipose tissues, muscles and internal organs of ruminants. In numerous studies it has been found that dietary vegetable oils rich in polyunsaturated fatty acids (PUFA) decreased the SFA concentrations in the tissues of ruminants and increased the concentrations of monounsaturated fatty acids (MUFA) and PUFA, including conjugated linoleic acid (CLA) isomers (Cho and Kim, 2011; Kupczyński *et al.,* 2011; Czauderna *et al.,* 2012). Furthermore, adding fish oil (FO) added to diets has been shown to increase the concentrations of CLA isomers and long-chain PUFA (LPUFA), particularly EPA (C20:5n-3), DPA (C22:5n-3) and DHA (C22:6n-3), in ruminant tissues (Wood *et al*., 2008; Buccioni *et al.,* 2012). Clinical studies have demonstrated that n-3LPUFAs (like EPA, DPA and DHA) possess anti-arrhythmic, anti-thrombotic and anti-inflammatory properties. Recent studies showed that dietary CLA isomers suppress chemically induced tumour development in animal models. Diets enriched with carnosic acid or FO (rich n-3LPUFA) also improved immune responses, exerted anti-inflammatory effects and increased CLA concentrations in animal tissues (Wąsowska *et al.,* 2006; Cho and Kim, 2011; Ibarra *et al.,* 2011). Unfortunately, many studies have indicated that increases in PUFA content (particularly LPUFA) in tissues stimulates oxidative stress in animals and humans (González and Tejeda, 2007). Considering these facts, adequate levels of health-promoting PUFA in the diets of ruminants combined with antioxidant compounds (such as rosemary extract, tocopherols or Se-compounds) is crucial for maintaining healthy farm animals (Navarro-Alarcon and Cabrera-Vique, 2008; Jordan *et al.,* 2013; Morán *et al.,* 2013; Dragoev *et al.,* 2014). In this context, special attention has been paid to phenolic diterpenes from *Rosmarinus officinalis* (*R. officinalis*). In fact, *R. officinalis* carnosic acid (CA) has been reported to have neuroprotective and anti-aging effects and has also been shown to prevent amyotrophic lateral sclerosis (Ibarra *et al.,* 2011; Sasaki *et al.,* 2013). Dietary CA has the ability to modify ruminal microbiota and hence the biosynthesis of volatile compounds and fatty acid metabolism in the rumen (Morán *et al.,* 2012a, 2013). Other studies investigating CA have documented improvements in meat quality. Dietary CA seems to extend the shelf life of lamb meat (Morán *et al.,* 2012a,b, 2013). Furthermore, recent studies have shown that dietary CA protected unsaturated fatty acids (UFA), particularly LPUFAs, in animal tissues from peroxidation damage (Morán *et al.,* 2012b, 2013; Jordan *et al.,* 2013). Similarly, Se-compounds (especially Se-enzymes) can remove reactive oxygen species and radicals in animal models (Juniper *et al.,* 2008; Yu *et al.,* 2008). Maintenance of appropriate

AA (arachidonic acid; C20:4n-6) metabolism, lowering the levels of peroxides and free radicals within cells, and decreasing oxidative stress and peroxidative damage to PUFA in animal tissues are all important physiological functions of half of the Se-cysteine-containing enzymes (Se-Cys-enzymes) (Navarro-Alarcon and Cabrera-Vique, 2008; Yu *et al.,* 2008; Ahmad *et al.,* 2012). In fact, PUFA oxidation produces toxic carbonyl products from lipid peroxidation in the meat and adipose tissues of farm animals. One such product is malondialdehyde (MDA), which is considered an on-invasive index of lipid peroxidation (Czauderna *et al.,* 2011). Numerous studies indicated that organic dietary forms of Se (such as selenized-yeast; SeY) or inorganic Se forms (such as selenite or selenate, SeVI) affected the levels of fatty acids (FA), tocopherols, cholesterol and its metabolites (e.g., cholest-4-en-3-one or its 3-oxo derivatives),and MDA in animal tissues (Suzuki *et al.,* 1998; Yu *et al.,* 2008; Xiao *et al.,* 2009; Czauderna *et al.,* 2012; Rozbicka-Wieczorek *et al.,* 2014a,b).

Based on these findings, we hypothesized that adding FO, CA and Se (as SeY or SeVI) to diets can significantly modify FA and tocopherol levels, and modulate oxidative stress in the adipose tissues of lambs when compared to lambs fed a diet with only rapeseed oil (RO) (rich in n-6PUFA). Therefore, the first objective of our study was to investigate the effects of the FO-supplemented diet containing RO on the levels of FA, tocopherols, total cholesterol (TCh), cholesterol metabolites (such as cholest-4-en-3-one or its 3-oxo derivatives), non-enzymatic cholesterol oxidation products (Ch-Ox) and MDA in the subcutaneous adipose tissue (SAT) of lambs and compare them to the SAT of lambs fed the diet with only RO. The second objective of our study was to investigate the effects diets supplemented with different chemical forms of Se (as SeY or SeVI) including RO, FO and CA on the contents of FA, tocopherols, TCh, cholesterol metabolites, Ch-Ox and MDA in the SAT and compare them to the SAT of lambs fed RO- and FO-containing diets, irrespective of the presence of CA.

## MATERIALS AND METHODS

**Animals, housing, diets, experimental design and sampling:** Thirty male Corriedale lambs with an average body weight (BW) of 30.5±2.6 kg at the beginning of the experiment were individually penned and divided into 5 treatment groups of 6 animals. The animals were distributed into 5 groups, according to the initial weight of the lambs such that the average initial body weights were similar between groups. The study was conducted under the authority of the Third Local Commission of Animal Experiment Ethics at the University of Life Sciences (Ciszewskiego 8, 02-786 Warsaw, Poland). During a 3-week preliminary period the animals were given free access to the standard concentrate-hay diet with vitamins and mineral premix (the basal diet; Table1). The basal diet (BD) consisted of the following components: meadow hay (~36%), a mixture of soybean meal (~36%) and barley meal (~16.5%), wheat starch (~9%) and mineral-vitamin mixture (20 $g \cdot kg^{-1}$ BD). This BD contained 120 g of crude protein, 12 g of crude fibre and 11 MJ of metabolizable energy in 1 kg dry matter. The Se content of the BD was 0.16 ppm.

The BD was supplemented with either 3% RO (the control diet) or 2% RO and 1% odourless FO (Tables 2 and 3). After the preliminary period, the lambs were fed either the BD containing 3% RO (the control diet; Group I), the BD enriched with 2% RO, 1% FO (the ROFO diet; Group II) or the ROFO diet enriched with 0.1% CA with or without 0.35 ppm Se as SeY or SeVI (Table 3) for 35 days. All diets were adjusted weekly and supplied as two equal meals at 07:30 and 16:00 each day to ensure free access to the feed. The di*et al*lowance was changed weekly according to the lamb's body weight. All lambs were fed the same quantity of freshly prepared diets with one of the following additives: RO, FO, CA, SeY or SeVI. The additives were added to the concentrate daily and then vigorously mixed (Table3). The animals ate the entire meal. The average daily diet intake was 1.08 kg per lamb. Fresh drinking water was always available. The lambs were slaughtered at the end of the 35-day experiment (i.e., at 07:00-08:00 h) after 12 hrs of fasting. The lambs were rendered unconscious via an intramuscular injection of xylazine (2-4 mg/10 kg BW).The animals were slaughtered in accordance with the European Union Council Regulations (EC) No 1099/2009 dated 24.09.2009 for the protection of animals at the time of slaughter in small experimental slaughterhouses. SAT samples were harvested and frozen. Homogenized SAT samples were stored in sealed tubes at -32°C until analysis.

**Chemicals:** Acetonitrile, methanol and n-hexane were purchased from Lab-Scan (Dublin, Ireland).Other reagents were of analytical grade (Avantor Performance Materials, Gliwice, Poland). CLA isomer mixture and other fatty acid standards, 25% $BF_3$ in methanol and sodium selenate (SeVI) were provided by Sigma (St. Louis, MO, USA). Dichloromethane, methanol, KOH, NaOH and concentrated HCl were purchased from Avantor Performance Materials (Gliwice, Poland). The organic solvents were of HPLC grade. Carnosic acid (CA) was purchased from Hunan Geneham Biomedical Technology Ltd. (Changsha, Hunan, China). RO and odourless FO were supplied by Company AGSOL (Pacanów, Poland).The vitamin and mineral mixture was purchased from POLFAMIX OK (Grodzisk Mazowiecki, Poland). The selenized yeast (Se-*Saccharomyces cerevisiae*; SeY) was donated by Sel-Plex (Alltech Inc.,St. Louis, MO, USA). Approximately 83% of the

total Se-content of the SeY is in the form of seleno-methionine (Se-Met). 5% is in the form of seleno-cysteine (Se-Cys) incorporated into the proteins of *Saccharomyces cerevisiae* (Rayman, 2004).

**Analytical methods and chromatographic equipment:** SAT samples (15–20 mg) were saponificated using KOH solutions according to methods described by Rozbicka-Wieczorek *et al.*(2014a).Then, base- and acid-catalysed methylations were introduced for the preparation of methyl esters of fatty acids (FA) in processed biological samples (Rozbicka-Wieczorek *et al.,* 2014a). Methylated fatty acids (FAME) in the fat tissue samples were then quantified using capillary gas chromatography with mass spectrometry (GC-MS) according to methods described by Rozbicka-Wieczorek *et al.* (2014a). The FAME analyses were performed on a SHIMADZU GC-MS-QP2010 Plus EI equipped with a BPX70 fused silica column (120 m × 0.25 mm i.d. × 0.25 µm film thickness; Phenomenex, Torrance, CA, USA), a quadruple mass selective detector (Model 5973N) and an injection port. FAME identification was validated based on electron impact ionization spectra of FAME and compared to authentic FAME standards and the NIST 2007 reference mass spectra library. All FAME analyses performed on the SAT samples were based on total ion current (TIC) chromatograms and/or selected-ion monitoring (SIM) chromatograms.

The tocopherols and TCh were quantified in SAT samples using a reversed-phase liquid chromatographic system (SHIMADZU, Tokyo, Japan) according to methods described by Czauderna *et al.* (2009). The liquid chromatographic instrument used consisted of an ultra-fast liquid chromatography system (UFLC-DAD), incorporating two LC-20ADXP pumps (UFLCXR), a SIL-20ACXR auto sampler (LFLCXR), a CBM-20A communications bus module, a CTO-20A column oven, a DGU-20A5 degasser and a SPD-photodiode array detector (DAD).

Ch-Ox products were determined in the SAT samples using capillary GC-MS according to methods described by Czauderna *et al*. (2013). The MDA contents in the SAT samples were determined after saponification followed by derivatisation according to methods described by Czauderna *et al*. (2011). The chromatographic separations of derivatized MDA from endogenic species of the SAT samples were conducted using UFLC-DAD (Czauderna*et al.,* 2011).Contents of FA, tocopherols, TCh and MDA in the SAT samples were expressed from fresh matter.

**Statistical analyses:** Statistical analyses were performed using the STATISTICA ver. 10 PL software package (StatSoft Inc.; Tulsa, OK, USA). Differences among dietary treatments (FO, CA and Se as SeVI or SeY) were tested using Tukey's post hoc test. The results are presented as the mean values of the individually analysed SAT samples. Polynomial contrasts were also used to determine linear effects of the diets enriched with FO, CA and/or Se (as SeY or SeVI) on the contents of fatty acids, TCh, tocopherols and MDA in the SAT. Significance was determined at a $P$ value $\leq 0.05$. Trends were considered when $0.05 < P \leq 0.10$. All values shown represent the group means and the standard errors of the mean (SEM).

## RESULTS

**The effects of experimental diets on body weight, feed conversion efficiency and SFA profile in the SAT:** In the current study, neither macroscopic lesions nor pathological changes were observed in the internal organs, muscles or adipose tissues of lambs fed the control and supplemented diets. The results summarized in Table 3show that the CASeVI diet increased the lambs' body weight (BW) and feed conversion efficiency (FCE) when compared to lambs fed the control, CA and CASeY diets. The ROFO diet tended to increase ($0.05 < P \leq 0.1$) the lambs' BW and FCE when compared to lambs fed the control diet. Our studies demonstrated the influence of the experimental diets enriched with either FO, CA, SeVI or SeY on the concentration of SFA in the SAT of lambs (Table4). All experimental diets decreased (contrast <0.01) the concentrations of C15:0 and C18:0 in the SAT when compared to the control diet. Similarly, the ROFO diet reduced ($P \leq 0.05$; contrast $\leq 0.05$) the concentrations of C12:0, C14:0, C15:0, C16:0 and C18:0 in the SAT when compared to the control. The CASeVI diet decreased ($P \leq 0.05$; contrast $\leq 0.05$) the concentrations of C10:0, C12:0, C14:0, C15:0, C16:0, C18:0 and C22:0 in the SAT when compared to the CASeY diet. The CASeVI diet decreased ($P \leq 0.05$) the concentrations of atherogenic SFA (A-SFA), thrombogenic SFA (T-SFA), the concentration sums of SFA ($\sum$SFA) and medium-chain SFA ($\sum_{medium}$SFA) in the SAT when compared to the CA and CASeY diets (contrast $\leq 0.03$) and the control diet. Consequently, the atherogenic index ($_{index}A^{SFA}$) was lower ($P \leq 0.05$) in the SAT of lambs fed the CASeVI diet when compared to the SAT of lambs fed the CA and CASeY diets (contrast $\leq$ 0.039) and the control diet. The SAT of lambs fed the CASeY diet had the highest concentration ($P \leq 0.05$) of $\sum_{medium}$SFA. All experimental diets reduced (contrast < 0.0001) the ratios of $\sum$SFA to the concentration sums of UFA ($\sum$SFA/$\sum$UFA) and to all FA assayed ($\sum$SFA/$\sum$FA) in the SAT when compared to the control.

**The effects of experimental diets on the concentration of unsaturated fatty acids in the SAT:** Our study demonstrated the influence of the experimental diets enriched with FO, CA or Se (as SeVI or SeY) on the concentrations of MUFA and PUFA in the SAT of lambs (Tables 5 and 6). Our results showed that adding CA

with/without SeY to the diet containing RO and FO increased (P≤ 0.05) the concentration sum of MUFA (ΣMUFA) when compared to ROFO and CASeVI diets (contrast ≤ 0.02) (Table 5). The ROFO, CASeY and CASeVI diets decreased (*P*≤ 0.05) the concentration of *t11*C18:1 in the SAT when compared to the control and CA diets. The diets containing SeY or SeVI increased (P≤ 0.05) the Δ9-desaturase (Δ9-index) and ΣΔ9-desaturase index (ΣΔ9-index) in the SAT when compared to the control, ROFO and CA diets. Moreover, all experimental diets increased (contrast <0.001) the *c11*C20:1 concentration, Δ9-index and ΣΔ9-index in the SAT when compared to the control diet. The CASeY and CASeVI diets produced the highest concentrations of *c11*C20:1 (P≤ 0.05) in the SAT.

This study demonstrates that the CASeVI diet decreased (P≤ 0.05) the concentrations of *c9t11*CLA and other *ct/tc*CLA isomers (Σ*ct/ct*CLA) as well as the sum of all CLA isomers (ΣCLA) in the SAT when compared to the ROFO diets (Table 6). Moreover, the concentrations of Σ*ct/ct*CLA and ΣCLA were lower (P≤ 0.05) in the SAT of lambs fed the CASeY diet when compared to lambs fed the control and ROFO diets. All experimental diets decreased (contrast = 0.001) the concentrations of Σ*ct/ct*CLA and ΣCLA in the SAT when compared to the control diet.

The concentrations of C18:2n-6 (LA) and C18:3n-3 (αLNA), the sum of PUFA (ΣPUFA), including Σn-6PUFA and Σn-3PUFA, were lower in the SAT of lambs fed the CASeVI diet than in CASeY diet-fed lambs (P≤ 0.05; contrast ≤ 0.008). The concentrations (P≤ 0.05; contrast ≤ 0.05) of AA, DPA, DHA and the sum of LPUFA (ΣLPUFA), including Σn-6LPUFA and Σn-3LPUFA, were higher in the SAT of lambs fed the ROFO diet than in the control diet-fed lambs. It has been found that the CA, CASeY and CASeVI diets decreased (P≤ 0.05) the concentrations of ∑LPUFA, including ∑n-6LPUFA, ∑n-3LPUFA, AA and DPA in the SAT when compared to the ROFO diet. The SeVI diet increased (P≤ 0.05) the concentration ratios of ∑n-6PUFA to ∑n-3PUFA (∑n-6/∑n-3) and Δ4-desaturase index (Δ4-index) in the SAT when compared to the control and the other experimental diets. All experimental diets increased (P≤ 0.05; contrast ≤ 0.02) the concentration ratio of ∑n-3LPUFA to ∑FA (∑n-3LPUFA/∑FA), the fatty acid elongase index ($Elong_{index}$) and the Δ4-index in the SAT relative to the control diet.

**The effects of experimental diets on the concentration of TCh, cholesterol derivatives, tocopherols and MDA in the SAT:** Our study demonstrated that the CASeVI diet increased (P≤ 0.05) the concentrations of TCh relative to the control and the other experimental diets (Table 7). In contrast, adding FO to the RO diet did not change (P> 0.05) the concentrations of TCh, δ-tocopherol (δT), α-tocopherol (αT), α–tocopherol acetate (αTAc) and the sum of all assayed tocopherols (∑Ts) in SAF when compared to the control diet. The CASeY and CASeVI diets increased (P≤ 0.05) the concentrations of δT, γ-tocopherol (γT) and αT relative to the control, ROFO and CA diets. The concentrations (P≤ 0.05) of αT and ∑Ts were higher in the SAT of lambs fed the experimental diets containing CA, irrespective of the presence of SeY or SeVI, when compared to the control and ROFO diets.

**Table 1. Chemical composition (%) of the concentrate-hay diet (the basal diet) with vitamins and mineral mixture[1] fed to lambs**

| Item | Meadow hay[3] | Concentrate[2] | | |
|---|---|---|---|---|
| | | Barley meal | Soybean meal | Wheat starch |
| Dry matter | 88.4 | 87.6 | 89.7 | 87.3 |
| Crude protein | 9.50 | 9.94 | 41.81 | 0.90 |
| Crude fibre | 27.29 | 2.87 | 4.34 | - |
| Crude fat | 3.40 | 2.50 | 2.25 | 0.09 |
| Ash | 4.85 | 1.84 | 6.16 | 0.12 |
| Neutral detergent fiber | 59.17 | 18.02 | 18.81 | - |
| Acid detergent fiber | 32.08 | 4.61 | 6.44 | - |
| Acid detergent lignin | 4.47 | 1.14 | 1.49 | - |

[1] 1 kg of the mineral and vitamin mixture comprised: 285 g Ca, 16 g phosphorus, 56 g Na, 42 mg Co as carbonate, 10 mg iodine as iodate, 1 g Fe as sulphate, 6 mg Se as selenite, 0.5 g Cu as sulphate, 5.8 g Mn as sulphate, 7.5 g Zn as sulphate; vitamins: A (500 000 IU/kg), D3 (125 000 IU/kg), and E as α-tocopherol (25 000 IU/kg). 20 g of the mineral and vitamin mixture was added to 1 kg of the basal diet (BD). [2] The gross energy (MJ per kg of dry matter (DM)): barley meal: 16.3, soybean meal: 17.8, wheat starch: 16.7. [3] The gross energy: 17.1 MJ per kg of DM.

Our study demonstrated that the concentrations of cholesterol metabolites (such as cholest-4-en-3-one or its 3-oxo derivatives) (Suzuki *et al.,* 1998; Xiao *et al*., 2009) and cholesterol non-enzymatic oxidation products (Ch-Ox) in the SAT of lambs fed the control and experimental diets were below the detection limit ($L_D$). In

other words, lambs' SAT did not contain any observable amounts of cholesterol metabolites or Ch-Ox.

In our study, the experimental diets did not affect the MDA concentrations in the SAT (Table 7). MDA concentration did increase (P≤ 0.05) in the SAT of ROFO diet-fed lambs when compared to control, CA and CA SeVI diet-fed lambs. Similarly, lambs fed the ROFO diet had a higher (P≤ 0.05; contrast ≤ 0.04) PUFA peroxidation index ($MDA_{index}$) in the SAT when compared to control or CA diet-fed lambs. In contrast, lambs fed the CA diet had the lowest concentration (P≤ 0.05) of MDA in the SAT when compared to control diet-fed lambs and lambs fed the other experimental diets. Adding SeY or SeVI to the experimental diet (Groups IV and V) increased (P≤ 0.05) the $MDA_{index}$ in the SAT when compared to CA diet-fed (contrast ≤ 0.003) and control diet-fed lambs.

**Table 2**. **The concentrations (mg/kg) of the main fatty acids (FA) in the components of the lambs' diet: concentrate, meadow hay, rapeseed oil (RO) and odourless fish oil (FO).**

| Fatty acids | Concentrate | Meadow hay | RO | FO[1] |
|---|---|---|---|---|
| C8:0 | - | 83 | - | - |
| C12:0 | - | 142 | - | 82 |
| C14:0 | 104 | 239 | 56 | 12345 |
| C15:0 | - | - | - | 477 |
| *c9*C14:1 | - | 131 | - | 215 |
| C16:0 | 3189 | 4034 | 13091 | 56947 |
| *c7*C16:1 | - | - | - | 318 |
| *c*9C16:1 | - | 184 | 33 | 420 |
| ∑C16:2 | - | - | - | 15586 |
| C17:0 | - | - | - | 493 |
| *c9*C17:1 | - | - | - | 193 |
| C18:0 | 1425 | 459 | 5490 | 9452 |
| *c*6C18:1 | - | - | 6 | 188 |
| *c7*C18:1 | - | - | - | 842 |
| *c*9C18:1 | 774 | 1266 | 385859 | 290592 |
| *c12*C18:1 | - | 72 | 786 | 15834 |
| *c14*C18:1 | - | - | - | 159 |
| C18:2n-6(LA) | 29163 | 13100 | 3282394 | 114512 |
| C18:3n-3(αLNA) | 1014 | 4178 | 83474 | 20968 |
| *c7c9c12c15*C18:4 | - | - | - | 473 |
| C20:0 | - | 58 | 430 | - |
| *c11*C20:1 | - | 74 | - | 24206 |
| *c11c14*C20:2 | - | - | - | 2270 |
| *c8c11c14*C20:3 | - | - | - | 258 |
| C20:4n-6(AA) | - | - | - | 304 |
| *c8c11c14c17*C20:4 | - | - | - | 607 |
| C20:5n-3(EPA) | - | - | - | 6792 |
| C22:0 | - | 101 | 153 | 139 |
| *c11*C22:1 | - | - | - | 1704 |
| *c13*C22:1 | - | - | - | 11036 |
| *c13c16*C22:2 | - | - | - | 95 |
| *c7c10c13c16*C22:4 | - | - | - | 144 |
| C22:5n-3(DPA) | - | - | - | 1560 |
| C22:6n-3(DHA) | - | - | - | 26570 |
| C24:0 | - | 69 | - | - |
| *c15*C24:1 | - | 71 | 61 | 397 |

[1] The iodine value of FO: 50-65 g/100 g FO; the acid value of FO: 20 mg KOH/g FO.

**Table 3. The experimental scheme and the composition of the control diet (the RO diet) and the experimental diets, the body weight (BW), the body weight gain (BWG) and the feed conversion efficiency (FCE) of lambs[1].**

| Group | Additives added to the basal diet (BD) | The body weight of lambs | | BWG[4] kg | FCE[5] kg/kg |
|---|---|---|---|---|---|
| | | $BW_{initial}$[2] kg | $BW_{35days}$[3] kg | | |
| Group I[6] (The control group) | 3% RO (The RO diet[8]) | 30.7±2.5$^{a}$ | 36.3±2.8$^{a}$ | 5.6±1.0$^{a\alpha}$ (0.16±0.03) | 0.150±0.001$^{a\alpha}$ |
| Group II[7] | 2% RO and 1% FO (The ROFO diet[8]) | 30.6±2.1$^{a}$ | 37.7±1.8$^{ab}$ | 7.1±1.0$^{ab\beta}$ (0.20±0.03) | 0.189±0.001$^{ab\beta}$ |
| Group III[7] | 2% RO, 1% FO and 0.1% CA (The CA diet[8]) | 30.6±1.9$^{a}$ | 37.2±1.4$^{a}$ | 6.6±0.8$^{a}$ (0.19±0.02) | 0.174±0.001$^{a}$ |
| Group IV[7] | 2% RO, 1% FO, 0.1% CA and 0.35 ppm Se as SeY (The CASeY diet[8]) | 30.3±1.3$^{a}$ | 36.8±1.4$^{a}$ | 6.5±0.7$^{a}$ (0.19±0.02) | 0.174±0.001$^{a}$ |
| Group V[7] | 2% RO, 1% FO, 0.1% CA and 0.35 ppm Se as Se VI (The CASeVI diet[8]) | 30.3±2.1$^{a}$ | 38.5±2.7$^{b}$ | 8.2±0.7$^{b}$ (0.23±0.02) | 0.215±0.001$^{b}$ |

[1] Data are presented as means and the corresponding root-mean-square error (RMSE) of the means. Significance was declared at $^{a,b}P \leq 0.05$ and trends – when at $0.05 < ^{\alpha,\beta}P \leq 0.10$.
[2] The average initial body weight (kg) of lambs after the 3-week of the preliminary period.
[3] The average body weight (kg) of lambs fed the diets for 35 days of the experimental period.
[4] $BWG = (BW_{35days} - BW_{initial})$; in parentheses: the average daily body weight gain (BWG/35).
[5] kg body weight gain/kg diet intake.
[6] For the 3-week of preliminary period lambs were fed the diet with 3% RO.
[7] For the 3-week of preliminary period lambs were fed the diet with 2% RO and 1% FO.
[8] The concentration of Se in the RO, ROFO and CA diets was 0.16 ppm; the concentration of Se in the CASeY and CASeVI diets was 0.51 ppm.

**Table 4. The concentrations (mg/g SAT) of selected individual saturated fatty acids (SFA), atherogenic-SFA[1] (A-SFA), thrombogenic-SFA[2] (T-SFA), the sums of medium SFA ($\Sigma_{medium}$ SFA)[3], all assayed SFA (ΣSFA)[4] and the ratios of ∑SFA to the sum of PUFA (ΣSFA/∑PUFA), UFA (∑SFA/ΣUFA) and all assayed FA (∑SFA/∑FA) in the SAT of lambs.**

| Specification | I | II | III | IV | V | SEM | Contrast[5] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I/II | II/III | III/IV | III/V | IV/V | I/Exp |
| C10:0 | 1.38$^{a}$ | 1.25$^{a}$ | 1.57$^{b}$ | 1.62$^{b}$ | 1.10$^{a}$ | 0.06 | 0.10 | 0.001 | 0.34 | 0.01 | 0.007 | 0.48 |
| C12:0 | 1.93$^{a}$ | 1.02$^{b}$ | 2.40$^{c}$ | 2.79$^{c}$ | 0.89$^{d}$ | 0.16 | 0.02 | 0.002 | 0.12 | 0.002 | 0.0001 | 0.33 |
| C14:0 | 23.9$^{a}$ | 18.3$^{b}$ | 25.8$^{c}$ | 32.4$^{d}$ | 15.5$^{b}$ | 1.18 | 0.05 | 0.002 | 0.02 | 0.002 | 0.0001 | 0.35 |
| C15:0 | 1.90$^{a}$ | 1.39$^{b}$ | 1.44$^{b}$ | 1.46$^{b}$ | 0.96$^{c}$ | 0.07 | <0.0001 | 0.27 | 0.40 | 0.02 | 0.018 | <0.0001 |
| C16:0 | 155$^{a}$ | 148$^{b}$ | 160$^{a}$ | 169$^{c}$ | 134$^{d}$ | 3 | 0.012 | 0.002 | 0.007 | 0.02 | 0.006 | 0.40 |
| C17:0 | 11.2$^{a}$ | 10.7$^{a}$ | 11.1$^{a}$ | 11.1$^{a}$ | 9.3$^{a}$ | 0.4 | 0.37 | 0.40 | 0.69 | 0.17 | 0.17 | 0.29 |
| C18:0 | 211$^{a}$ | 174$^{b}$ | 179$^{b}$ | 170$^{b}$ | 140$^{c}$ | 6 | 0.01 | 0.17 | 0.11 | 0.04 | 0.05 | 0.002 |
| C22:0 | 0.088$^{a}$ | 0.078$^{a}$ | 0.156$^{b}$ | 0.103$^{a}$ | 0.092$^{a}$ | 0.010 | 0.33 | 0.004 | 0.056 | 0.03 | 0.36 | 0.30 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A-SFA | 180[a] | 167[b] | 188[a] | 204[c] | 151[b] | 4 | 0.01 | 0.001 | 0.005 | 0.01 | 0.001 | 0.39 |
| $_{index}A^{SFA\ 6}$ | 0.678[a] | 0.614[ac] | 0.648[b] | 0.716[d] | 0.618[c] | 0.011 | 0.11 | 0.027 | <0.001 | 0.039 | <0.001 | 0.17 |
| T-SFA | 390[a] | 340[a] | 366[a] | 372[a] | 310[b] | 9 | <0.001 | 0.001 | 0.051 | 0.03 | 0.02 | 0.02 |
| $\Sigma_{medium}$SFA | K27.3[ab] | 20.7[ad] | 29.6[b] | 36.8[c] | 17.5[d] | 1.5 | 0.051 | 0.003 | 0.008 | 0.003 | <0.0001 | 0.39 |
| ΣSFA, | 406[a] | 355[ab] | 382[a] | 389[a] | 303[b] | 9 | <0.001 | <0.001 | 0.067 | 0.03 | 0.02 | 0.02 |
| ΣSFA/ΣPUFA | 7.62[a] | 7.38[ab] | 7.85[a] | 6.99[b] | 7.37[ab] | 0.08 | 0.06 | <0.0001 | 0.0002 | 0.001 | 0.01 | 0.05 |
| ΣSFA/ΣUFA | 1.079[a] | 0.972[b] | 0.931[b] | 0.911[b] | 0.923[b] | 0.012 | <0.0001 | 0.057 | 0.24 | 0.35 | 0.47 | <0.0001 |
| ΣSFA/ΣFA | 0.517[a] | 0.489[b] | 0.481[b] | 0.477[b] | 0.478[b] | 0.003 | <0.0001 | 0.053 | 0.25 | 0.37 | 0.46 | <0.0001 |

[1] The sum: C12:0, C14:0 and C16:0.
[2] The sum: C14:0, C16:0 and C18:0.
[3] The sum: C8:0, C10:0, C12:0 and C14:0.
[4] The sum: C6:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0 and C24:0.
[5] Least squares means for concentrations of selected SFA in the SAT of lambs fed the control and experimental diets (the groups: I-V); I/II = I vs. II; II/III = II vs. III; III/IV = III vs. IV; III/V = III vs. V; IV/V = IV vs. V; I/Exp = I vs. all experimental groups (II-V).
[6] The atherogenic index = (C12:0 + 4 x C14:0 + C16:0) / (ΣMUFA + Σn-6PUFA + Σn-3PUFA) (Morán *et al.,* 2013).

**Table 5**. **The concentrations (mg/g SAT) of selected individual monounsaturated fatty acids (MUFA), the sums of positional *c*C18:1 isomers (Σ*c*C18:1)[1] and all assayed MUFA (ΣMUFA) [2] and index values of Δ9-desaturase (Δ9-index [3]) and ΣΔ9-desaturase (ΣΔ9-index [4]) in the SAT of lambs.**

| Specification | I | II | III | IV | V | SEM | Contrast[5] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I/II | II/III | III/IV | III/V | IV/V | I/Exp |
| *c9*C14:1 | 4.28[a] | 3.37[ab] | 3.67[a] | 3.98[a] | 2.80[b] | 0.10 | 0.001 | 0.045 | 0.02 | 0.11 | 0.055 | <0.001 |
| *c7*C16:1 | 5.83[a] | 8.32[b] | 11.24[bc] | 12.95[c] | 6.55[ab] | 0.61 | <0.001 | 0.005 | 0.07 | 0.03 | 0.006 | 0.005 |
| *c9*C16:1 | 6.27[a] | 5.55[a] | 6.26[a] | 6.70[a] | 6.24[a] | 0.13 | 0.04 | 0.01 | 0.06 | 0.48 | 0.21 | 0.40 |
| *c9*C17:1 | 2.65[ab] | 2.25[a] | 2.77[ab] | 3.16[b] | 2.67[ab] | 0.09 | <0.01 | 0.013 | 0.055 | 0.41 | 0.15 | 0.40 |
| *c10*C17:1 | 0.59[a] | 0.59[a] | 0.93[b] | 0.77[ab] | 0.60[a] | 0.04 | 0.48 | 0.002 | 0.06 | 0.07 | 0.18 | 0.11 |
| *c6*+*c7*C18:1 | 27.5[a] | 29.3[a] | 25.0[a] | 30.4[b] | 19.3[a] | 1.2 | 0.27 | 0.08 | 0.01 | 0.15 | 0.02 | 0.35 |
| *c9*C18:1 | 263[abc] | 252[bc] | 288[ab] | 295[a] | 236[c] | 5 | 0.01 | 0.002 | 0.21 | 0.02 | 0.007 | 0.33 |
| *c12*C18:1 | 9.9[a] | 11.8[a] | 14.7[b] | 14.5[b] | 10.4[a] | 0.4 | 0.008 | 0.006 | 0.41 | 0.005 | 0.01 | 0.004 |
| *c11*C20:1 | 0.85[a] | 1.35[bc] | 1.27[b] | 1.54[d] | 1.51[cd] | 0.1 | 0.001 | 0.13 | 0.001 | 0.001 | 0.13 | 0.001 |
| *t11*C18:1 | 2.53[a] | 2.05[b] | 2.57[a] | 1.85[bc] | 1.38[c] | 0.11 | 0.03 | 0.02 | 0.005 | 0.01 | 0.12 | 0.06 |
| Σ*c*C18:1 | 300[ab] | 293[ab] | 327[ab] | 340[b] | 265[a] | 9 | 0.30 | 0.046 | 0.08 | 0.04 | 0.02 | 0.21 |
| ΣMUFA | 323[ac] | 317[a] | 362[c] | 371[c] | 287[a] | 7 | 0.20 | <0.001 | 0.17 | 0.02 | 0.01 | 0.26 |
| Δ9-index | 0.562[a] | 0.600[b] | 0.616[c] | 0.635[d] | 0.641[d] | 0.02 | 0.01 | 0.10 | 0.11 | 0.19 | 0.41 | 0.001 |
| ΣΔ9-index | 0.553[a] | 0.590[b] | 0.606[b] | 0.633[c] | 0.626[c] | 0.02 | 0.02 | 0.05 | 0.04 | 0.21 | 0.45 | 0.001 |

[1] The concentration sum: *c6*C18:1, *c7*C18:1, *c9*C18:1 and *c12*C18:1.
[2] The concentration sum: ∑*c*C14:1, ∑*c*C15:1, ∑*c*C16:1, ∑*c*C16:1, ∑*c*C17:1, ∑*t*C18:1, ∑*c*C18:1, *c*11C20:1, ∑*c*C22:1 and *c*15C24:1.
[3] Δ9-index = *c9*C18:1 / (*c9*C18:1 + C18:0).
[4] ΣΔ9-index= (*c9t11*CLA + *c9*C18:1) / (*c9t11*CLA + *c9*C18:1 + *t11*C18:1 + C18:0).
[5] Least squares means for concentrations of selected MUFA in the SAT of lambs fed the control and experimental diets (the groups: I-V); I/II = I vs. II; II/III = II vs. III; III/IV = III vs. IV; III/V = III vs. V; IV/V = IV vs. V; I/Exp = I vs. all experimental groups (II-V).

**Table 6. The concentrations (mg/g) of *c9t11*CLA, other *ct/ct*CLA isomers (Σ*ct/tc*CLA)[1], the sum of all CLA isomers (ΣCLA)[2], selected individual PUFA, the sums of n-6PUFA (∑n-6PUFA)[3], n-3PUFA (∑n-3PUFA)[4], n-6LPUFA (∑n-6LPUFA)[5], n-3LPUFA (∑n-3LPUFA)[6], LPUFA (∑LPUFA)[7], all assayed PUFA (∑PUFA)[8], the ratios of ∑n-6PUFA to ∑n-3PUFA (Σn-6/Σn-3), ∑PUFA to ∑SFA (∑PUFA/∑SFA), ∑PUFA to ΣFA[9] (∑PUFA/∑FA) and ∑n-3LPUFA to ∑FA (∑n-3LPUFA/∑FA)[10],values of the fatty acid elongase index (Elong$_{index}$)[11] and the Δ4-desaturase indexes (Δ4-index)[12] in the SAT of lambs.**

| Specification | I | II | III | IV | V | SEM | Contrast[13] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I/II | II/III | III/IV | III/V | IV/V | I/Exp |
| *c9t11*CLA | 1.42$^{ab}$ | 1.63$^{a}$ | 1.54$^{a}$ | 1.32$^{ab}$ | 1.16$^{b}$ | 0.10 | 0.06 | 0.001 | 0.051 | 0.048 | 0.29 | 0.49 |
| Σ*ct/tc*CLA | 1.59$^{a}$ | 0.56$^{b}$ | 0.25$^{c}$ | 0.16$^{c}$ | 0.21$^{c}$ | 0.10 | <0.001 | 0.012 | 0.19 | 0.36 | 0.22 | 0.001 |
| ΣCLA | 3.02$^{a}$ | 2.19$^{b}$ | 1.79$^{bc}$ | 1.48$^{c}$ | 1.37$^{c}$ | 0.11 | 0.001 | 0.006 | 0.045 | 0.13 | 0.43 | 0.001 |
| LA | 45.6$^{ab}$ | 41.8$^{ac}$ | 42.9$^{ab}$ | 49.6$^{b}$ | 37.2$^{c}$ | 1.7 | 0.13 | 0.17 | 0.001 | 0.08 | 0.006 | 0.20 |
| αLNA | 4.08$^{a}$ | 3.13$^{b}$ | 3.24$^{b}$ | 3.85$^{a}$ | 1.80$^{c}$ | 0.04 | 0.16 | 0.13 | 0.005 | 0.003 | 0.001 | 0.005 |
| AA | 376$^{a}$ | 433$^{b}$ | 307$^{c}$ | 337$^{ac}$ | 330$^{ac}$ | 1.2 | 0.05 | 0.01 | 0.12 | 0.40 | 0.47 | 0.32 |
| DPA | 0.228$^{a}$ | 0.339$^{b}$ | 0.225$^{a}$ | 0.244$^{a}$ | 0.227$^{a}$ | 0.015 | 0.01 | 0.001 | 0.19 | 0.49 | 0.41 | 0.23 |
| DHA | 0.037$^{a}$ | 0.070$^{cd}$ | 0.060$^{bc}$ | 0.049$^{ab}$ | 0.084$^{d}$ | 0.006 | 0.01 | 0.24 | 0.29 | 0.05 | 0.04 | 0.05 |
| Σn-6PUFA | 46.0$^{a}$ | 42.2$^{a}$ | 43.2$^{ab}$ | 49.9$^{a}$ | 37.5$^{b}$ | 0.1 | 0.13 | 0.24 | 0.001 | 0.09 | 0.006 | 0.20 |
| Σn-3PUFA | 4.34$^{a}$ | 3.54$^{b}$ | 3.53$^{b}$ | 4.15$^{ab}$ | 2.11$^{c}$ | 0.11 | 0.003 | 0.46 | 0.01 | 0.001 | 0.002 | 0.01 |
| Σn-6LPUFA | 0.376$^{a}$ | 0.432$^{b}$ | 0.307$^{c}$ | 0.337$^{ac}$ | 0.330$^{ac}$ | 0.018 | 0.08 | 0.04 | 0.12 | 0.40 | 0.47 | 0.31 |
| Σn-3LPUFA | 0.266$^{a}$ | 0.410$^{b}$ | 0.285$^{a}$ | 0.312$^{a}$ | 0.311$^{a}$ | 0.020 | <0.001 | <0.001 | 0.28 | 0.39 | 0.50 | 0.10 |
| ΣLPUFA | 0.642$^{a}$ | 0.842$^{b}$ | 0.592$^{a}$ | 0.649$^{a}$ | 0.641$^{a}$ | 0.060 | 0.05 | 0.04 | 0.39 | 0.48 | 0.36 | 0.26 |
| ΣPUFA | 53$^{ba}$ | 48$^{ba}$ | 49$^{ba}$ | 56$^{b}$ | 41$^{a}$ | 1 | 0.08 | 0.29 | 0.01 | 0.07 | 0.008 | 0.09 |
| Σn-6/Σn-3 | 10.6$^{a}$ | 11.9$^{a}$ | 12.2$^{a}$ | 12.0$^{a}$ | 17.8$^{b}$ | 1.6 | <0.001 | 0.08 | 0.23 | 0.009 | 0.009 | 0.01 |
| ∑PUFA/∑SFA | 0.129$^{a}$ | 0.141$^{b}$ | 0.129$^{a}$ | 0.141$^{b}$ | 0.138$^{b}$ | 0.001 | 0.01 | <0.001 | <0.001 | <0.01 | <0.001 | 0.06 |
| ∑PUFA/∑FA | 0.0677$^{a}$ | 0.0686$^{b}$ | 0.0617$^{c}$ | 0.0677$^{a}$ | 0.0660$^{d}$ | <0.001 | <0.001 | <0.001 | 0.002 | 0.012 | 0.002 | 0.001 |
| ∑n-3LPUFA/∑FA | 0.368$^{a}$ | 0.653$^{b}$ | 0.378$^{c}$ | 0.384$^{c}$ | 0.493$^{d}$ | 0.009 | <0.001 | <<0.001 | 0.35 | 0.005 | 0.005 | 0.01 |
| Elong$_{index}$ | 0.0007$^{a}$ | 0.0034$^{b}$ | 0.0035$^{b}$ | 0.0014$^{c}$ | 0.0021$^{c}$ | 0.0003 | 0.048 | 0.39 | 0.002 | 0.01 | 0.09 | 0.02 |
| Δ4-index | 0.144$^{a}$ | 0.172$^{b}$ | 0.211$^{c}$ | 0.169$^{b}$ | 0.271$^{d}$ | 0.005 | 0.04 | 0.03 | 0.02 | 0.01 | 0.003 | <0.001 |

[1] The sum of *ct/tc*CLA isomers: *cis-trans*CLA: *11-13, 12-14*; *trans-cis*CLA: *7-9, 8-10, 9-11, 10-12,11-13* and *12-14.*
[2] The sum: *c9t11*CLA, *ct/tc*CLA isomers, *tt*CLA isomers (*trans-trans*: 7-7, *8-10, 9-11, 10-12, 11-13* and *12-14*) and *cc*CLA isomers (*cis-cis*: *8-10, 9-11, 10-12* and *c11-12*).
[3] The sum: LA, *c6c9c12*C18:3 and Σn-6LPUFA.
[4]The sum: αLNA, *c6c9c12c15*C18:4 and Σn-3LPUFA.
[5] The sum: *c*11*c*14C20:2, *c*8*c*11*c*14C20:3, AA, *c*13*c*16C22:2 and *c7c*10*c*l3*c*16C22:4.
[6]The sum: *c*11*c*14*c*17C20:3, *c*8*c*11*c*14*c*17C20:4, EPA, DPA and DHA.
[7] The sum: Σn-6LPUFA and Σn-3LPUFA.
[8] The sum:*c*9*c*12C16:2, *c6c*9*c*12C16:3, ΣCLA, Σn-3PUFA and Σn-6PUFA.
[9] The sum of all assayed fatty acids (ΣFA).
[10] The concentration ratio of ∑n-3LPUFA (μg/g SAT) to ∑FA (mg/g SAT).
[11] Elong$_{index}$ = C20:2n-6 / (C20:2n-6 + LA).
[12] Δ4-index = DHA / (DHA + DPA).
[13] Least squares means for concentrations of selected MUFA in the SAT of lambs fed the control and experimental diets (the groups: I-V); I/II = I vs. II; II/III = II vs. III; III/IV = III vs. IV; III/V = III vs. V; IV/V = IV vs. V; I/Exp = I vs. all experimental groups (II-V).

**Table 7. The concentrations of total cholesterol (TCh), δ–, γ– and α–tocopherol (δT, γT and αT), α–tocopheryl acetate (αTAc), MDA and values of the PUFA peroxidation index ($MDA_{index}$) in the SAT of lambs fed the control and experimental diets.**

| Specification | I | II | III | IV | V | SEM | Contrast[1] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | I/II | II/III | III/IV | III/V | IV/V | I/Exp |
| TCh, μg/g | 52.8$^{ab}$ | 59.3$^{a}$ | 47.7$^{b}$ | 50.5$^{ab}$ | 69.9$^{c}$ | 0.21 | 0.23 | <0.001 | 0.04 | 0.01 | 0.02 | 0.33 |
| δT, μg/g | 0.76$^{a}$ | 0.70$^{a}$ | 0.85$^{a}$ | 1.37$^{b}$ | 1.44$^{b}$ | 0.10 | 0.29 | 0.08 | 0.001 | 0.05 | 0.41 | 0.07 |
| γT, μg/g | 0.94$^{a}$ | 0.66$^{b}$ | 0.89$^{ab}$ | 1.23$^{c}$ | 1.11$^{c}$ | 0.08 | 0.04 | 0.17 | 0.04 | 0.04 | 0.31 | 0.30 |
| αT, μg/g | 1.64$^{a}$ | 1.90$^{a}$ | 2.23$^{b}$ | 2.96$^{c}$ | 2.61$^{c}$ | 0.10 | 0.02 | 0.04 | 0.05 | 0.04 | 0.21 | 0.02 |
| αTAc, μg/g | 4.58$^{ab}$ | 4.17$^{a}$ | 4.65$^{b}$ | 4.99$^{b}$ | 4.63$^{b}$ | 0.20 | 0.25 | 0.04 | 0.33 | 0.45 | 0.31 | 0.41 |
| ΣαT[2], μg/g | 6.22$^{a}$ | 6.07$^{a}$ | 6.88$^{ab}$ | 7.95$^{b}$ | 7.24$^{b}$ | 0.33 | 0.48 | 0.26 | 0.18 | 0.41 | 0.26 | 0.12 |
| ΣTs[3], μg/g | 7.92$^{a}$ | 7.44$^{a}$ | 8.62$^{b}$ | 10.56$^{b}$ | 9.80$^{b}$ | 0.35 | 0.04 | 0.04 | 0.13 | 0.35 | 0.31 | 0.01 |
| MDA, ng/g | 2.92$^{a}$ | 5.07$^{b}$ | 1.74$^{c}$ | 4.51$^{b}$ | 3.18$^{a}$ | 0.10 | 0.02 | 0.004 | <0.001 | 0.001 | 0.005 | 0.07 |
| $MDA_{index}$[4] | 0.055$^{ab}$ | 0.106$^{c}$ | 0.035$^{b}$ | 0.081$^{c}$ | 0.078$^{c}$ | 0.02 | 0.04 | 0.003 | 0.002 | 0.003 | 0.41 | 0.27 |

[1] Least squares means for concentrations of selected MUFA in the SAT of lambs fed the control and experimental diets (the groups: I-V); I/II = I vs. II; II/III = II vs. III; III/IV = III vs. IV; III/V = III vs. V; IV/V = IV vs. V; I/Exp = I vs. all experimental groups (II-V).

[2] The concentration sum of αT and αTAc.

[3] The concentration sum of δT, γT, αT and αTAc.

[4] The concentration ratio of MDA (ng/g) to ΣPUFA (mg/g); $MDA_{index}$ = MDA / ∑PUFA.

# DISCUSSION

**The effects of experimental diets on feed conversion efficiency and body weight of lambs:** The primary objective of the current study was to test the possibility that CA with or without Se (as SeY or SeVI) could decrease oxidative stress and peroxidative damage of UFA in the SAT of lambs. In fact, similar to all other fat organs, the SAT is an active part of the endocrine system, secreting resistin and leptin hormones (Marieb and Hoehn, 2008). Moreover, the SAT conserves internal body heat by providing a layer of insulation and protects the internal organs and bones. Fortunately, FO, CA, SeY and SeVI did not produce any macroscopic lesions or toxic symptoms in adipose fat tissues, muscles or internal organs of lambs fed the experimental diets (Rozbicka-Wieczorek *et al.,* 2016a,b).

The current study demonstrated that adding FO to the RO diet (Group II) tended to increase the FCE and BW of lambs ($0.05 < P \leq 0.10$) more than the control diet. In fact, it has been reported that adding n-3LPUFA in the form of FO to animal diets enhanced anabolic potential and reduced muscle loss (Ewaschuk *et al.,* 2014). Furthermore, low plasma concentrations of n-3LPUFA (like EPA or DHA) have been associated with skeletal muscle reduction in animals and humans (Bhullar *et al.,* 2016). Moreover, our results demonstrated that adding SeVI to the diet containing RO, CA and FO significantly increased FCE and BW in lambs ($P \leq 0.05$) when compared to control diet-fed lambs and lambs fed the experimental diets with CA, irrespective of the presence of SeY. Indeed, dietary SeVI (via selenide) can be used to synthesize Se-Cys, which is inserted into seleno-enzyme's primary structure (Ahmad *et al.,* 2012; Raymond *et al.,* 2014). These Se-Cys containing enzymes stimulate thyroid hormone synthesis and regulate important biochemical reactions, particularly protein synthesis and enzymatic capacity that accompany increases in metabolic rate. Considering the results described above, we argue that adding SeVI to diets containing RO, FO and CA most effectively stimulate anabolic processes in lambs. On the other hand, the experimental diet supplemented with SeY (rich in Se-Met) did not affect ($P > 0.05$) FCE or BW in lambs (Group IV) when compared to control, ROFO and CAdiet-fed lambs. Indeed, Se-Met derived from SeY is less reactive. Because $tRNA_{Met}$ does not discriminate between Se-Met and methionine (Met), dietary Se-Met replaces Met in proteins (Navarro-Alarcon and Cabrera-Vique, 2008; Thiry *et al*., 2012). Therefore, these Se-Met-containing proteins are not considered seleno-proteins (Raymond *et al.,* 2014). In contrast to the Se-Cys containing enzymes, the effects of Se-Met-containing proteins on thyroid hormone synthesis were shown to be negligible; however, they are important in regulating certain biochemical reactions, particularly protein synthesis (Raymond *et al.,* 2014). In fact, Se-Met as a protein residue is a stable and safe way to store Se in the bodies of animals and humans (Navarro-Alarcon and Cabrera-Vique, 2008; Thiry *et al.,* 2012; Raymond *et al*., 2014).

**Effect of the experimental diets on fatty acid composition in the SAT:** The present study demonstrates that the experimental diets (particularly the CASeVI diet) reduced the concentrations of C15:0 and C18:0, and increased the Δ9- and Δ4-desaturase indexes (Δ9-index, ΣΔ9-index and Δ4-index) in the SAT when compared control diet-fed lambs (Tables 4-6). Interestingly, the experimental diets containing SeY or SeVI more effectively increased the Δ9-index and ΣΔ9-index in the SAT than the control, ROFO and CA diets. As consequence, the CASeY and CASeVI diets decreased the *t11*C18:1 concentration when compared to the control and CA diets. In light of these findings, we conclude that the present results are consistent with our previous studies showing that dietary SeY or SeVI increased Δ9-desaturation capacity in the adipose tissues and muscles of animal models (Czauderna *et al.,* 2003; Rozbicka-Wieczorek *et al.,* 2014b). Thus, we argue that SeY and particularly SeVI added to diets (Groups IV and V) stimulated the capacity of Δ9-desaturase in the adipose tissues of lambs. We propose that supplementation in Groups IV and V increased Δ9-desaturase capacity via stimulation of stearoyl-CoA desaturase mRNA expression in the SAT. Consequently, the experimental diet containing only CA did not effectively increase ($P \leq 0.05$) Δ9-desaturase capacity in the SAT when compared to experimental diets including SeY or SeVI. Moreover, our study demonstrated that adding CA to the diet with RO and FO (Group III) increased Δ9-desaturation capacity when compared to the control and ROFO diets.

Additionally, the experimental diet including SeVI most effectively increased ($P \leq 0.05$) the capacity of Δ4-desaturase in the SAT. As consequence, the CASeVI diet increased the DHA concentration in the SAT when compared to the control, CA and CASeY diets (Table 6). In fact, recent studies have shown that the DHA concentration and Δ4-desaturase activity were positively correlated with the concentration of Se-dependent enzymes and hormones (Quilliot *et al.,* 2003; Ahmad *et al*., 2012). Conversely, the experimental diet with SeY (rich in Se-Met) had the weakest impact (contrast = 0.003) on the Δ4-desaturase index (Δ4-index) in the SAT when compared to the CASeVI diet. Therefore, when compared to the CASeVI diet, the CASeY diet decreased ($P \leq 0.05$; contrast = 0.04) the DHA concentration in the SAT. In fact, dietary SeY (rich in Se-Met) stimulated the biosynthesis of Se-Met-containing proteins, which had no impact on important biochemical reactions, particularly protein or enzyme biosynthesis (Raymond *et al.,* 2014).

In this study, we demonstrated that the experimental diet contained SeVI reduced ΣSFA concentrations, including $\sum_{medium}$SFA and A-SFA, in the SAT when compared to the control diet. In fact, the current study showed that the CASeVI diet stimulated the Δ9- and Δ4-desaturation of fatty acids in the SAT more strongly than the control diet. Moreover, the current study and our previous on rats (Czauderna *et al.,* 2003) showed that compared to the control and CASeY diets, the CASeVI diet more effectively reduced (via β-oxidation) ∑SFA concentrations (particularly $\sum_{medium}$SFA) than ∑PUFA concentrations (especially ∑n-3LPUFA, such as DPA or DHA) in animal tissues. Consequently, the ratio of Σn-3LPUFA/ΣFA was higher in the SAT of CASeVI diet-fed lambs than in the control and other experimental groups. Based these findings, we argue that the CASeVI diet improved the nutritional quality of the lambs' SAT as an ingredient for food production. Presumably, similar changes in fatty acid profiles also occur in intramuscular fat; however, this assumption requires verification. Conversely, the experimental diet containing SeY (rich in Se-Met) stimulated biosynthesis of Se-Met-containing proteins, which do not affect the β-oxidation of fatty acids in the SAT. The concentrations of ΣSFA, ΣMUFA and ΣPUFA were higher in the SAT of CASeY diet-fed lambs ($P \leq 0.05$; contrast ≤ 0.02) than in lambs fed the CASeVI diet.

In our study, the concentration of C18:0 (the product of the terminal biohydrogenation of UFA) and the ratio of ∑SFA/∑UFA and ∑SFA/∑FA in the SAT of lambs fed the experimental diets decreased when compared to the control diet (contrast≤ 0.002). Thus, our results are in agreement with recent studies published by Wąsowska *et al.* (2006) who also found that dietary FO (rich in EPA and DHA) decreased the capacity of ruminal bio-hydrogenation of UFA to the more saturated FA. In fact, the addition of EPA or DHA to pure cultures inhibited the isomerase activity and growth of *B. fibrisolvens*. Consequently, dietary FO inhibited the biohydrogenation of LA and αLNA causing the accumulation of a number of intermediates in ruminal digesta and ruminant tissues. Interestingly, the CASeY diet significantly increased the accumulation of LA and αLNA in the SAT when compared to the ROFO diet. These results clearly demonstrate that dietary SeY (rich in Se-Met) decreased the metabolism of LA and αLNA in the rumen and tissues of lambs. Moreover, our recent study showed that the CASeY diet most effectively increased LA and αLNA concentrations in the incubated ruminal fluids and muscles of lambs (Rozbicka-Wieczorek *et al.,* 2014b). Considering these findings, we suggest that dietary SeY reduced the capacity for bacterial isomerisation of LA and αLNA in the rumen. In contrast, the CASeVI diet reduced LA and αLNA concentrations in the SAT (contrast ≤ 0.006; Table 6) and in the ruminal fluids and muscles of lambs when compared to the CASeY diet (Rozbicka-Wieczorek *et al.,* 2014b). Therefore, it seems likely that adding SeVI to the diet increased bacterial isomerisation of PUFA (e.g., LA or αLNA) in the lambs' rumen.

**Effect of the experimental diets on the contents of TCh, tocopherols and oxidative stress in the SAT:** This study demonstrates that the CASeVI diet increased ($P \leq 0.05$) the concentration of TCh in the SAT when compared to the control and other experimental diets (Table 7). Our results are consistent with findings reported by Stranges *et al.* (2010) in laboratory animals. In this study, Se-supplemented rats had higher liver triglyceride contents, which may provide a possible explanation for the lipogenic effects of diets high in Se. Furthermore, Se-Cys-containing enzymes and cholesterol biosynthesis are linked through the common use of isopentenyl pyrophosphate both for the biosynthesis of Sec-tRNA and for isoprenoid biosynthesis in the mevalonate pathway (Stranges *et al.,* 2010). Increases in Se (especially SeVI or selenite) are associated with increased total and non-HDL cholesterol levels. On the other hand, CA and CASeY diets reduced ($P \leq 0.05$; contrast ≤ 0.02) TCh concentrations in the SAT when compared to the CASeVI diet. In fact, dietary SeY (rich in Se-Met) stimulated the biosynthesis of Se-Met-containing proteins that are not Se-enzymes (Raymond *et al.,* 2014).Indeed, our results demonstrate that Se-Met-containing proteins have no impact on the accumulation of TCh in the SAT. Similarly, the CA diet quantitatively ($P > 0.05$) and statistically ($P \leq 0.05$) reduced the TCh content in the SAT when compared to the control and other experimental diets. This is consistent with previous studies (Ibarra *et al.,* 2011) showing that CA-rich dietary rosemary (*Rosmarinus officinalis* L.) leaf extract limited weight gain and decreased cholesterol levels in animal models. Moreover, in this study, there were no detectable (below the quantification limit) concentration s of cholesterol metabolites and Ch-Ox in the SAT of lambs fed the control and experimental diets. We argue that the capacity for metabolic transformation and oxidation of cholesterol in the SAT of lambs is considerably lower than in brain tissues (Rozbicka-Wieczorek *et al.,* 2016a).

As shown in Table7, diets containing CA and SeY or SeVI stimulated the accumulation of all forms of tocopherols. The concentration of ΣTs was higher ($P \leq 0.05$) in the SAT of lambs fed the CA, CASeY and CASeVI diets than in control and ROFO diet-fed lambs. Our findings demonstrate that the experimental diets with CA, particularly those containing Se (as SeY or SeVI) spared all forms of tocopherol in the SAT of lambs. In fact, CA and Se-enzymes derived from dietary SeY (particularly SeVI) are antioxidants. Biosynthesized Se-enzymes prevent the widespread chain reaction of UFA peroxidation in animal tissues (Ahmad *et al.,* 2012;

Raymond *et al.,* 2014). Se-enzymes (such as glutathione peroxidase) synergistically with tocopherols regulate lipid peroxidation in animal tissues (Brigelius-Flohé and Maiorino, 2013). Our results show that the experimental diet containing only CA (Group III) exhibited weaker tocopherol sparing effects in the SAT than the experimental diets supplemented with both CA and Se (as SeY and SeVI).

In the present study, the CA-only diet produced the strongest ($P \leq 0.05$) antioxidative activity in the SAT of lambs when compared to the other experimental diets (Table 7). The CA diet decreased ($P \leq 0.05$) MDA concentrations (the by-product of PUFA peroxidation) when compared to the control and the other experimental diets. Moreover, the $MDA_{index}$ was lower in the SAT CA diet-fed lambs than other experimental diets. These results are consistent with previous studies (Hernández-Hernández *et al.*, 2009; Morán *et al*., 2012a,b, 2013) that demonstrated that CA effectively inhibits lipid oxidation in the tissues of farm animals. CA has two reactive phenol groups in its aromatic ring. The phenolic group at the 11-position was found to contribute more significantly to its antioxidant activity (Masuda *et al.,* 2001). Therefore, CA and other phenolic diterpenes in the edible parts of carcasses have received increased attention due to their protective effects against oxidative deterioration of certain carcass components, such as PUFA (Morán *et al.,* 2012a,b).

As expected, the ROFO diet (i.e., without extra antioxidants) more effectively increased the MDA concentration in the SAT than control (low in LPUFA), CA and CASeVI diets, i.e., diets containing anti-oxidant(s). Indeed, the FO added to the RO diet (Group II) is rich in LPUFAs. Unfortunately, LPUFAs are highly prone to oxidation and produce a potentially deleterious amount of oxidation products, such as the lipid aldehydes4-hydroxy-2-alkenals and MDA (Czauderna *et al.,* 2011). Therefore, the ROFO diet significantly increased the MDA concentration and the $MDA_{index}$ value in the SAT when compared to the control diet containing only RO (low in LPUFA). Given these findings, adequate FO content combined with antioxidants, such as CA or SeVI (used for synthesis of antioxidative Se-enzymes), is crucial to reduce oxidative stress (Navarro-Alarcon and Cabrera-Vique, 2008; Stranges *et al.,* 2010). Surprisingly, we discovered that the experimental diet containing only CA most effectively reduced PUFA oxidation in the SAT. The CA diet decreased MDA concentrations and the $MDA_{index}$ in the SAT when compared to the other experimental diets (high in LPUFA).

On the other hand, diets supplemented with both CA and Se (as SeY and SeVI) increased PUFA oxidation in the SAT when compared to the CA diet. Se-compounds added to diets are now thought to be toxic due to their pro-oxidant ability to catalyse thiol oxidation and simultaneously generate superoxide ($O_2^{\bullet-}$). Cytotoxicity of dietary Se-compounds is due to the formation of the selenide anion or by diselenide reduction (Suzuki, 2005; Navarro-Alarcon and Cabrera-Vique, 2008; Stranges *et al.,* 2010). Formation of the selenide anion depends on the amounts of dietary and chemical Se-compounds added to diets. Moreover, pro-oxidative effects of dietary Se-compounds are due to the catalysis of hydrosulphide oxidation that inhibits protein biosynthesis (Navarro-Alarcon and Cabrera-Vique, 2008). We propose that supplementing diets with SeY and SeVI stimulates the biosynthesis of antioxidative Se-enzymes. Moreover, dietary SeVI and SeY stimulated selenide anion formation and/or hydrosulphide oxidation in the SAT (Suzuki, 2005). Therefore, the $MDA_{index}$ and the MDA concentrations in the SAT were more effectively increased by CASeY and CASeVI when compared to the CA diet. On the other hand, the CASeVI diet enriched in SeVI, the essential component for Se-enzyme biosynthesis (Suzuki, 2005), reduced MDA concentrations in the SAT when compared to the ROFO diet. The ROFO diet (high in LPUFA) does not contain extra antioxidants (such as CAor SeVI). Consequently, the CA diet decreased the $MDA_{index}$ and the MDA concentrations in the SAT of lambs more than the ROFO diet.

**Conclusion:** Our current study demonstrates that short-term supplementation of CA and Se (as SeY or SeVI) can be used to increase the concentration of tocopherols in the SAT of lambs. The CA diet most effectively reduced oxidative stress in the SAT. All experimental diets decreased the concentration of C18:0 (the product of terminal biohydrogenation) in the SAT when compared to the control diet. Adding FO to the RO diet positively changed the fatty acid profiles of the SAT as this diet increased concentrations of ∑LPUFA (including ∑n-3LPUFA) and the ratios of ΣPUFA/ΣFA and Σn-3LPUFA/ΣFA in the SAT the most. These changes in the fatty acid concentrations in the SAT are especially beneficial to human health. Our study demonstrated that changes to the essential components of the SAT depend on the additive used to supplement the lambs' diet. The current study provides important insights for nutritionists conducting studies to improve the nutritional quality of feed for ruminants and humans. Further experiments are necessary to determine if diets with higher CA concentrations as well as other vegetable oils and antioxidants (like α-tocopherol or lycopene) change the profiles of fatty acids and other essential components in ruminant meat and adipose tissues to make them healthier for human consumption.

**Acknowledgements:** This study was in part supported by the National Science Centre (NCN): Grant No. 2013/09/B/NZ9/00291 and by the statutory funds from The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland.

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